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Our File: PPCT16894

European Patent Office
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The Netherlands

Attention: International Preliminary Examining Authority
(Authorized Officer Brouns, G.)

Dear Sirs:

Applicant: **McGill University**
PCT No. : **PCT/CA2004/000998**
Title: **Non-Inbred Embryonic Stem Cells Having Good Developmental Potential**

This is in response to the Written Opinion of the International Searching Authority dated November 8, 2004.

Article 34 Amendments

In accordance with Article 34 PCT, please amend the present application as follows:

In the claims:

In claims 1 and 3 after "potential" insert --and successfully compete with pre-existing inner cell mass cells, and their derivatives, when injected into a normal blastocyst--.

In claim 4, replace "an X-linked" with --a--.

Replace claims 9 and 10 with new claims 9 – 11.

Renumber claims 11 and 12 as claims 12 and 13, and correct dependencies accordingly.

Cancel claim 13.

Add new claims 14 and 15.

Renumber claim 14 as claim 16; step (b), replace "crosses and backcrosses" with --at least one cross and at least one backcross--; step (c), after "step (b)" insert --; and (d) deriving embryonic stem cells from the inner cell masses of said blastocysts--.

Renumber claim 15 as claim 17; step (b), after "step (a)" insert --or an offspring of a subsequent generation--; step (c), replace "crosses and backcrosses" with --at least one cross and at least one backcross-- and delete "between"; step (d), after "step (c)" insert --; and (e) deriving embryonic stem cells from the inner cell masses of said blastocysts--.

Renumber claims 16 – 21 as claims 18 – 23 and correct dependencies accordingly.

Renumber claim 22 as claim 24; correct the dependency accordingly; and replace "transgenic" with --genetically modified--.

Add new claims 25 and 26.

Replacement claim pages 46 – 50, incorporating the above amendments, are submitted herewith.

REMARKS

The Applicant has amended the claims in order to more clearly and precisely define the claimed subject matter.

Support for the amendment to claims 1 and 3 can be found throughout the application as originally filed, for example, at page 10, lines 14 – 20 and the results provided in Examples 3 – 8 and 10.

Support for the amendment to claim 4 can be found, for example, at page 11, line 7 to page 12, line 13.

Support for the amendment to claims 9 and 10 and for step (c) of new claim 11, by which it is specified that the ES cells are introduced into a normal blastocyst or a tetraploid blastocyst or aggregated with one or more pre-implantation embryos, can be found, for example, at page 15, lines 11 – 5.

Support for new claims 11, 15 and 26 can be found throughout the application as originally filed, for example, at page 13, line 19 to page 14, line 3.

Support for new claims 14 and 25 can be found throughout the application as originally filed, for example, in original claim 13.

Support for the amendment to claims 14 and 15 (renumbered as claims 16 and 17) in which it is specified that the multiple generations of breeding include a combination of "at least one cross and at least one backcross" can be found, for example, in the description of the production of ES cells of the present invention in Example 2 (pages 20 to 23) and the pedigrees depicted in Figure 2. Further, support for the amendment to these claims to add the step of "deriving embryonic stem cells from the inner cell masses of said blastocysts" can be found, for example, in Example 1 (pages 17 to 20).

In view of the foregoing, the Applicant asserts that no new matter has been added by way of the present amendments.

The Examiner has objected to previous claims 3, 6 – 13 and 20 – 22 as lacking novelty under Article 33(2) PCT in view of Egan et al. (2001) *Proc. Natl. Acad. Sci. USA.* 98: 6209-6214 (identified as "D1").

D1 discloses embryonic stem (ES) cells derived from crosses between different inbred mouse strains and between one inbred strain and *Mus castaneous*. The resulting F_1 ES cell clones, when injected into tetraploid blastocysts and subsequently transferred to recipient females, can give rise to embryos and subsequently to viable mature mice that are derived predominantly from the ES cells. There is nothing in D1 that teaches or suggests that good developmental potential could be achieved if the F_1 ES cells were placed in direct competition with wild-type inner cell mass cells. D1 merely reports the outcome when F_1 ES cells are injected into tetraploid embryos, which are genetically limited in their capacity to generate embryonic tissues.

In contrast to D1, claims 3, 6 – 10, 12 – 14 and 22 – 24, which correspond with previous claims 3, 6 – 13 and 20 – 22, are directed toward non-inbred mouse ES cells, and method of making and using such ES cells, wherein the ES cells comprise alleles derived from at least three different inbred mouse strains, have good developmental potential and successfully compete with pre-existing inner cell mass cells, and their derivatives, when injected into a normal blastocyst. Accordingly, the Applicant asserts that the subject matter of claims 3, 6 – 10, 12 – 14 and 22 – 24 is novel in accordance with Article 33(2) PCT, since there is no teaching or suggestion in D1 of ES cells that contribute significantly to chimeras produced by injection of the ES cells into normal blastocysts.

The Examiner has also objected to claims 1 – 22 as lacking an inventive step under Article 33(3) PCT in view of the disclosure in Yagi et al. (1993) *Analytical Biochemistry* 214: 70-76 (identified as "D2"). In this case, the Examiner has taken the position that although document D2 merely discloses ES cells derived from a cross between two different inbred mouse strains that give rise to chimeras that are 100% ES cell derived, the use of three inbred strains to generate ES cells having maximal heterosis would have been obvious.

D2 discloses an ES cell line that apparently has high germline-differentiating potency. These F_1 ES cells are derived from a cross between two different inbred mouse strains and give rise to chimeras that are 100% ES cell derived upon injection of the ES cells into eight-cell stage embryos. As such the ES cells disclosed in D2 form excellent chimeras, but only when they are aggregated with 8-cell morula (i.e. at a stage prior to inner cell mass formation). In contrast, when injected into blastocysts, they contribute poorly to the resulting chimeras leading the authors to suggest that these cells "cannot adapt to or are rejected from the inner cell mass when introduced into blastocysts."

As noted above, the ES cells of the present invention have good developmental potential and successfully compete with pre-existing inner cell mass cells, and their derivatives, when injected into a normal blastocyst. When the ES cells of the present invention are injected into normal blastocysts (*i.e.*, not an eight-cell stage morula) the injected ES cells compete with the cells of the inner cell mass and the resulting mouse is entirely or almost entirely ES cell derived. Neither D1 nor D2 disclose ES cells having this ability. For example, in D2 the ES cells are shown to be unable to compete with host inner cell mass cells that have not been manipulated. This fundamental difference gives rise to the advantage of the ES cells of the present invention in that they can be injected directly into a blastocyst and it is not necessary to inject them into the early stage morula or into a manipulated blastocyst, *e.g.*, a tetraploid blastocyst.

Moreover, with respect to the methods for preparing the ES cells of the present invention, these methods include at least one backcross. This is in contrast to the F1, F2, etc. types of crosses used in D1 and D2 and provides an additional level of natural selection of advantageous alleles in the preparation of the ES cells of the present invention that is not achieved using the standard breeding techniques in the prior art.

In view of the foregoing, the Applicant respectfully asserts that the subject matter of claims 1 – 26 submitted herewith is inventive in accordance with Article 33(3) PCT. Reconsideration is respectfully requested.

The Examiner has raised additional objections to the claims under Article 6 PCT. In paragraph 4.1 of the Written Opinion of the International Searching Authority, the Examiner has suggested that the ES cell lines disclosed in the application are derived from only two inbred mouse strains rather than three inbred mouse strains. In making this objection, the Examiner has taken the position that the two 129 substrains used are, in essence, only a single inbred mouse strain. In fact, this is not the case. The substrains used are actually as different as many strains that are not classified as "substrains." Thus, a worker skilled in the art would readily appreciate that the ES cell lines derived from, for example, the C57BL/6 and two 129 substrains do include the alleles of three inbred mouse strains.

In paragraph 4.2, the Examiner has indicated that it is not clear what the essential technical features of the present invention are since the independent claims refer to ES cells having various combinations of characteristics. As noted above, the claims of the present application have been amended to further define the ES cells in terms of their ability to successfully compete with pre-existing host inner cell mass cells of a normal blastocyst. This essential technical feature of the claimed invention is present irrespective of whether the cells (i) are derived from either two or three inbred mouse strains; (ii) include a transgene docking site or not; or (iii) are created with or without additional (back)crosses following the initial cross between the inbred strains. These additional features identified by the Examiner are present, or not, in specific embodiments of the present invention and do not constitute essential elements of the invention.

In paragraph 4.3, the Examiner has suggested that the relative term "good developmental potential" lacks clarity and may be defined more precisely by the technical features that allow comparison. The claims of patent are to be read in view of the application as a whole and since the term "good developmental potential" is clearly defined in the Specification (see, for example, page 10, lines 2 – 13) as referring to the ability of the ES cells to contribute at a high percentage to the cells of a chimera produced from the ES cells. Further, the Examiner has suggested that the term

"transgene docking site" has no well recognized meaning in the art and may comprise any known sequence in the genome. In fact, the application includes a detailed description of what is meant by "a transgene docking site" in relation to the present invention (see, for example, page 11, lines 7 – 21). Accordingly, the Applicant respectfully asserts that claims 1, 2, 3, 10(b) and (d), 14, 15 and 17 do not require amendment in order to more clearly and precisely define the claimed subject matter.

In view of the foregoing comments and Article 34 amendments, the Applicant respectfully requests reconsideration of this application and issuance of a favourable International Preliminary Examination Report.

Yours very truly,

Stephanie R. White, Ph.D.
Patent Agent

SRW/srw
Enclosure

THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. A preparation of non-inbred mouse embryonic stem (ES) cells that comprise alleles derived from at least three different inbred mouse strains, wherein the ES cells have good developmental potential and successfully compete with pre-existing inner cell mass cells, and their derivatives, when injected into a normal blastocyst.

2. The non-inbred ES cell preparation according to claim 1, wherein the ES cells additionally comprise a transgene docking site.

3. A preparation of non-inbred mouse embryonic stem (ES) cells that comprise alleles derived from at least two different inbred mouse strains and a transgene docking site, wherein the ES cells have good developmental potential and successfully compete with pre-existing inner cell mass cells, and their derivatives, when injected into a normal blastocyst.

4. The non-inbred ES cell preparation according to claim 2 or 3, wherein the transgene docking site is a deletion mutant of a hypoxanthine phosphoribosyltransferase (HPRT) gene.

5. The non-inbred ES cell preparation according to claim 2 or 3, wherein the transgene docking site comprises a *loxP* site.

6. The non-inbred ES cell preparation according to any one of claims 1 – 5, wherein chimeras derived from the ES cells exhibit greater than 50% ES cell contribution.

7. The non-inbred ES cell preparation according to claim 6, wherein chimeras derived from the ES cells exhibit greater than 90% ES cell contribution.

8. The non-inbred ES cell preparation according to claim 7, wherein chimeras derived from the ES cells exhibit about 100% ES cell contribution.

9. A method for producing an ES cell-derived mouse comprising the steps of:

   (a) introducing a non-inbred mouse ES cell preparation according to any one of claims 1 - 8 into a normal mouse blastocyst or a tetraploid mouse

blastocyst or aggregating a non-inbred mouse ES cell preparation according to any one of claims 1 – 8 with one or more pre-implantation embryos under conditions that result in production of at least one embryo;

(b) transferring the resulting embryo(s) into an appropriate foster mother; and

(c) maintaining the foster mother under conditions that result in development of live offspring.

10. A method for producing an ES cell-derived, transgenic mouse comprising the steps of:

(a) introducing one or more transgenic sequences into non-inbred mouse ES cells of an ES cell preparation according to any one of claims 2 or 4 - 8;

(b) maintaining the ES cells under conditions that result in homologous recombination at the transgene docking site such that the one or more transgenic sequences are incorporated in the genome of the ES cells;

(c) introducing the resultant recombinant ES cells into normal blastocyst(s) or tetraploid blastocyst(s) or said recombinant ES cells with one or more pre-implantation embryos, under conditions that result in production of at least one embryo;

(d) transferring the resulting embryo(s) into an appropriate foster mother; and

(e) maintaining the foster mother under conditions that result in development of live offspring, wherein the ES cells have good developmental potential.

11. A method for producing an ES cell-derived, gene targeted mouse comprising the steps of:

(a) performing a genetic alteration or mutation of one or more genes or parts of genes in non-inbred mouse ES cells of an ES cell preparation according to any one of claims 1 - 8;

(b) maintaining the ES cells under conditions that result in homologous recombination such that the knock-out is incorporated in the genome of the ES cells;

(c) introducing the resultant recombinant ES cells into normal blastocyst(s) or tetraploid blastocyst(s) or said recombinant ES cells with one or more pre-implantation embryos, under conditions that result in production of at least one embryo;

(d) transferring the resulting embryo(s) into an appropriate foster mother; and

(e) maintaining the foster mother under conditions that result in development of live offspring, wherein the ES cells have good developmental potential.

12. The method according to any one of claims 9, 10 or 11, wherein the appropriate foster mother is, a pseudopregnant female mouse.

13. An ES cell-derived mouse that is prepared according to the method of any one of claims 9 – 12.

14. The mouse according to claim 13, which is a transgene bearing mouse.

15. The mouse according to claim 13, which is a genetically altered or mutated mouse.

16. A method for preparing mouse embryonic stem cells having good developmental potential that comprises the steps of:

(a) mating a female mouse of a first inbred mouse strain with a male mouse of a second inbred mouse strain, wherein the first and the second mouse strains are different;

(b) performing multiple generations of breeding including a combination of at least one cross and at least one backcross from offspring obtained from the mating between the female mouse and the male mouse in step (a);

(c) recovering blastocysts from a mouse obtained following the multiple generations of breeding performed in step (b); and

(d) deriving embryonic stem cells from the inner cell masses of said blastocysts.

17. A method for preparing mouse embryonic stem cells having good developmental potential that comprises the steps of:

(a) mating a female mouse of a first inbred mouse strain with a male mouse of a second inbred mouse strain, wherein the first and the second mouse strains are different;

(b) mating an offspring of the mating of step (a) or an offspring of a subsequent generation with a mouse of a third inbred mouse strain;

(c) performing multiple generations of breeding including a combination of at least one cross and at least one backcross from offspring obtained from the mating of step (b);

(d) recovering blastocysts from a mouse obtained following the multiple generations of breeding performed in step (c) and

(e) deriving embryonic stem cells from the inner cell masses of said blastocysts.

18. The method according to claim 16 or 17, wherein the multiple generations of breeding comprises a combination of 5 or 6 crosses and backcrosses.

19. The method according to any one of claims 16 – 18, wherein at least one of the inbred mouse strains contains a transgene docking site.

20. The method according to claim 19, wherein the transgene docking site is a deletion mutant of a hypoxanthine phosphoribosyltransferase (HPRT) gene.

21. The method according to claim 19, wherein the transgene docking site comprises a *loxP* site.

22. A non-inbred embryonic stem (ES) cell preparation obtained by the method of any one of claims 16 – 21.

23. Use of the ES cell preparation according to any one of claims 1 – 8 or 22 for producing an ES cell derived mouse.

24. Use of the ES cell preparation according to any one of claims 1 – 8 or 22 for producing an ES cell derived genetically modified mouse.

25. The use according to claim 24, wherein said genetically modified mouse is a transgenic mouse.

26. The use according to claim 24, wherein said genetically modified mouse comprises a genetic alteration or mutation.